

Final Report
(FY06 3rd Quarter-FY07 4th Quarter)

Project Title: Kentucky Rural Energy Supply Program

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Recipient: University of Louisville Research Foundation

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Louisville, KY 40292

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Subcontractors: University of Kentucky

Cost-Sharing Partners: University of Kentucky

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Final Report

Project Title: Differentiating Microbial Pathway and Membrane Adaptations for Enhanced Performance in Extreme Environments

Project Objective: The overall goal of this project is to quantify and differentiate the metabolic pathway flux and membrane fluidity changes of wild-type and ethanol-adapted *C. thermocellum* in response to controlled pressure and exogenous ethanol. This overall goal will be achieved by: quantifying metabolic pathway (Obj. 1) and membrane fluidity changes (Obj. 2) between the wild type and an ethanol-adapted strain of *C. thermocellum* as a function of hydrostatic pressure and exogenous ethanol; and correlate the pathway changes and membrane fluidity with environmental treatments to gain a mechanistic understanding of cellular adaptations in an ethanol-tolerant organism (Obj. 3).

Status:

1. Progress in Past Quarter:

The PI's met on February 3, February 17, March 2, March 29, 2006, however these were only planning meetings as the money had not been received by the University of Kentucky. The PI's also met on April 14, April 25, May 9, June 6, and June 20, 2006 for project planning/progress meetings. Beginning May 9th, two students and a post-doctoral scientist joined the project meetings. The PI's met on July 6, August 9, August 22, September 5, October 3, and October 24th, 2006, and the same frequency through 2007. Three students and a post-doctoral scientist were employed by the project.

Objective 1: Quantify metabolic pathway changes between the wild type and an ethanol-adapted strain of *C. thermocellum* as a function of exogenous ethanol.

1st Quarter: Some preliminary metabolite analysis has been performed. 2nd Quarter: Preliminary pressurized chemostat runs were conducted to coordinate the fermentation with the real-time sampling required by the mass spectroscopists. Successful fermentation runs have been performed in the pressurized chemostat, both at atmospheric pressure and at 3.5 MPa. Some preliminary metabolite analysis has been performed, and the graph is included below.

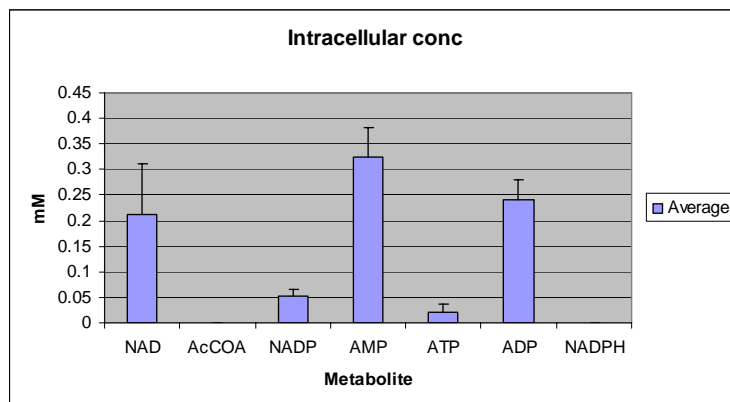


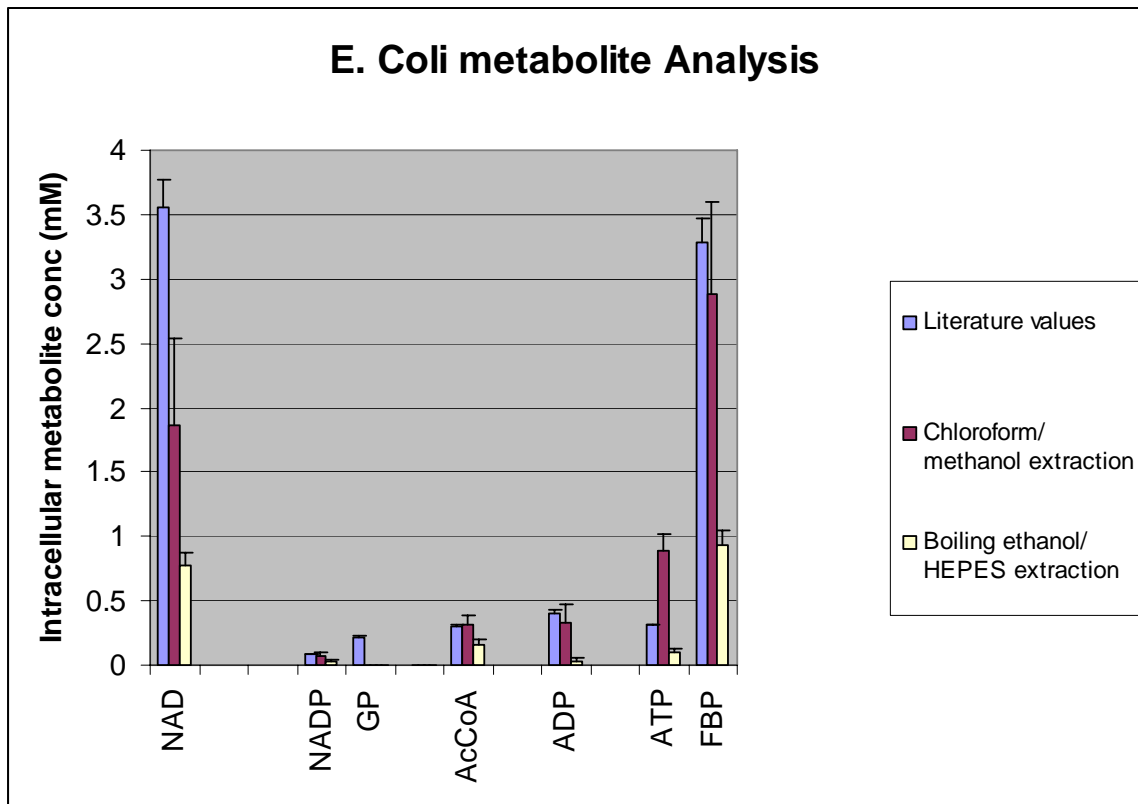
Figure 1: Intracellular metabolites measured from *C. thermocellum* (wild type) grown on cellobiose 4 g/L, in a chemostat with flow rate 16.7 ml/h, (Dilution rate 0.167 /h, Temp 60°C, Reactor volume 100 ml, No exogenous ethanol) at atmospheric pressure.

Graduate Student 2 (Chemistry): This student is working towards coupling capillary electrophoresis with the mass spec and/or UV. He has analyzed standards with CE, and found CE/MS wasn't as sensitive as CE/UV. He is using a new sheath liquid and ran CE/MS method again to check. Successfully separated peaks of 12 metabolite standards.

1. The intracellular concentrations we obtained for *C. thermocellum* were low by an order of magnitude compared to other organisms such as *E. coli*. To resolve this issue experiments were conducted to evaluate alternate extraction methods.

a) **Is CE-MS underestimating the concentration?**

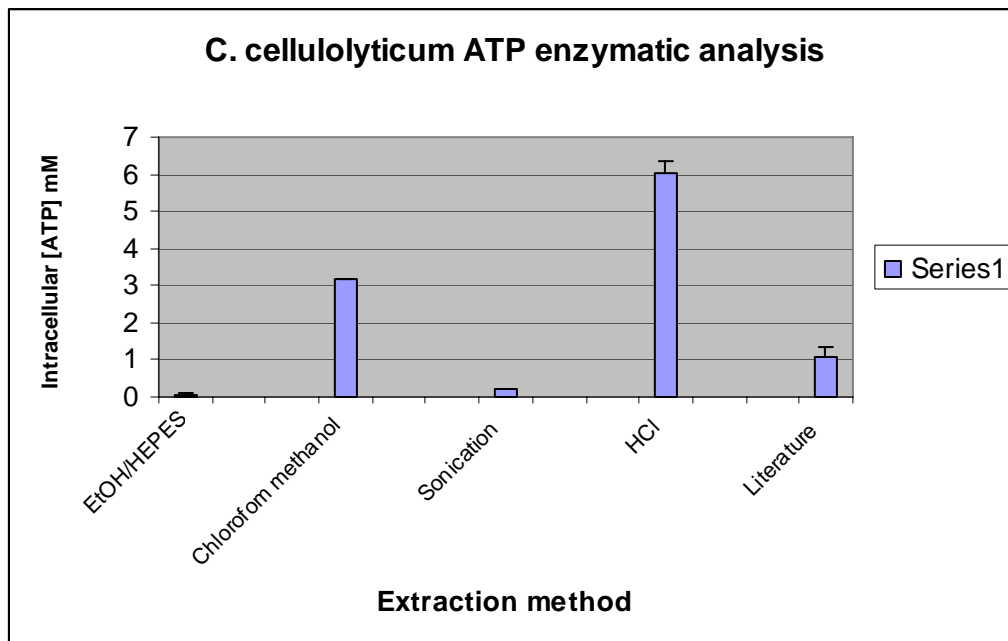
To evaluate this issue metabolite analysis was performed on *E. coli*.



- Metabolite concentrations obtained were the same order of magnitude as literature values.
- Chloroform/methanol extraction worked better than our old HEPES/ethanol extraction method.
- Low metabolite concentrations obtained for *C. thermocellum* were most likely due to the inefficiency of HEPES extraction method.

b) **Finding a better extraction method:**

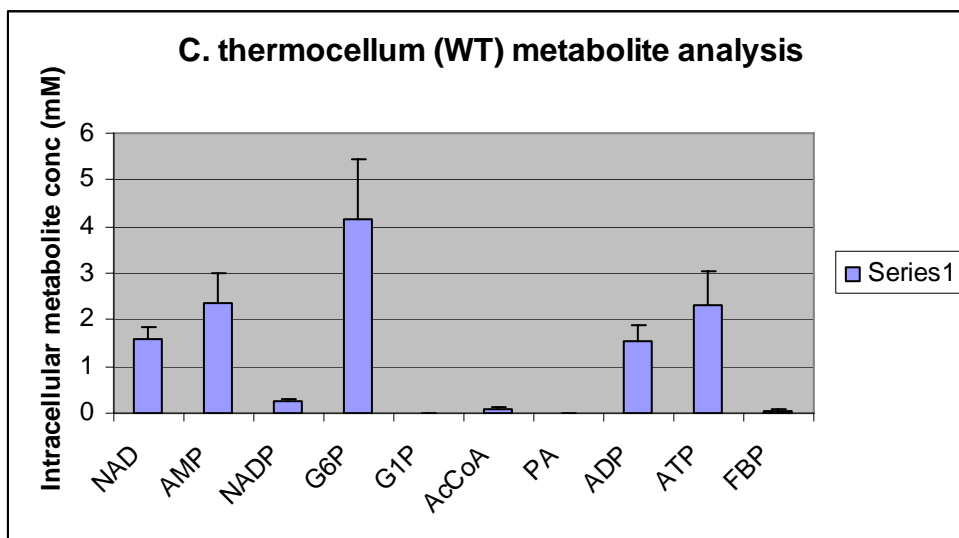
ATP was selected as a model metabolite to compare the extraction recovery from different extraction methods for *Clostridium cellulolyticum*. ATP was measured by enzymatic assay and obtained concentrations were compared with literature values.



- HCl extraction gave the highest ATP recovery
- The significant difference in HCl extraction to literature is mainly because of *C. cellulolyticum* cells were grown in batch culture as compared to chemostat cultures in the literature.

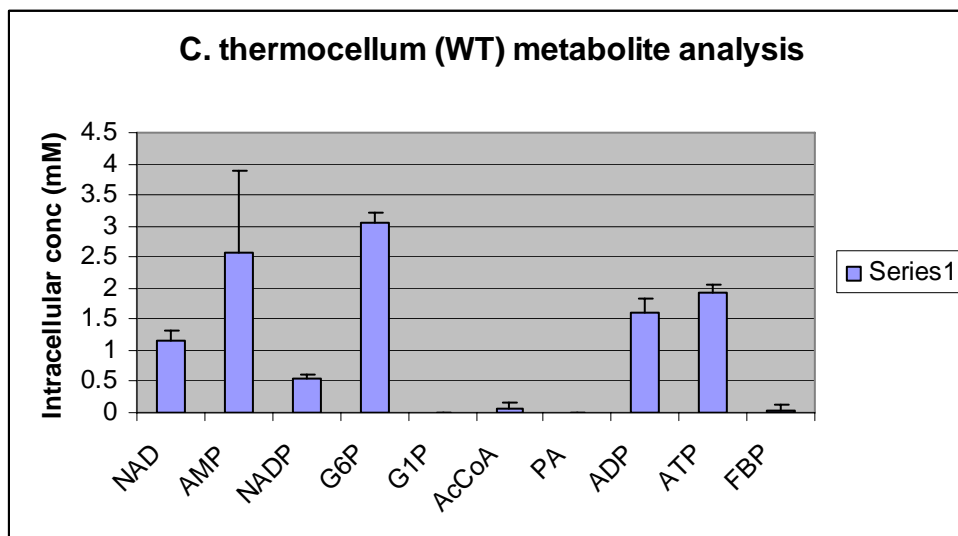
c) Development of CE compatible HCl extraction method:

A CE compatible HCl extraction method was developed and applied to *C. thermocellum* metabolite analysis.



- Recoveries of most of the metabolites improved with HCl extraction method, however it prevented analysis of pyruvic acid, NADH and NADPH.

- In addition, reproducibility of the data is an issue with this method.

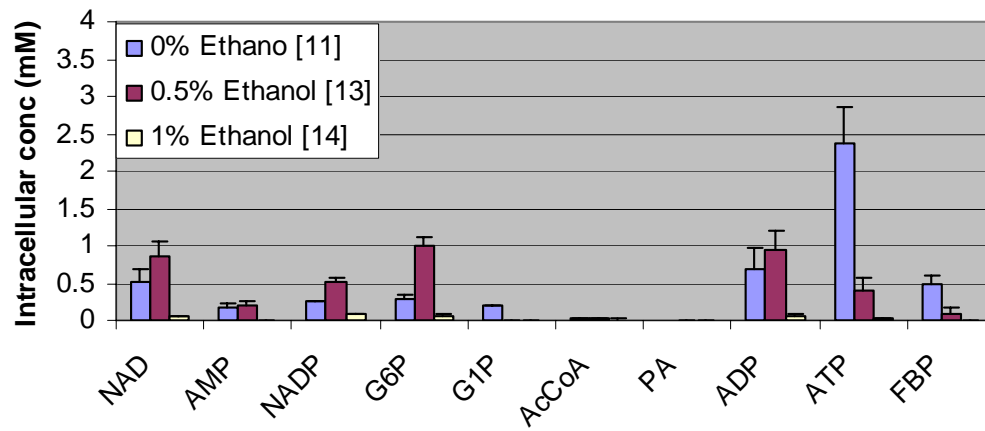


- Preliminary findings showed ultracold chloroform/methanol extraction method is as efficient as HCl extraction.
- This method showed less sample to sample analysis variation.
- The tested metabolites appeared to be stable under these extraction conditions.
- Reproducibility of this method will be tested.
- The reactor was operated at 0.1 MPa and different concentrations of ethanol. The CE/MS analyzed real data.

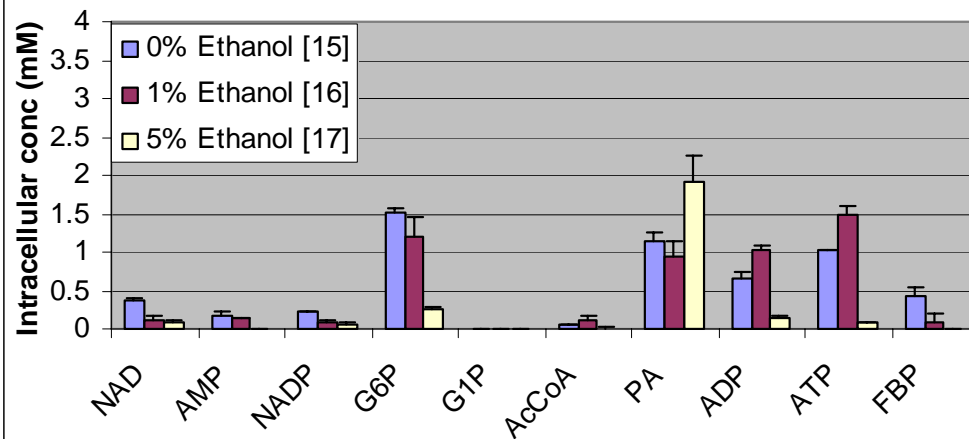
Actual Accomplishments:

Expt #	Cell type	% Ethanol
11	WT	0
13	WT	0.5
14	WT	1
15, 19	EA	0
16, 20	EA	1
17, 21	EA	5

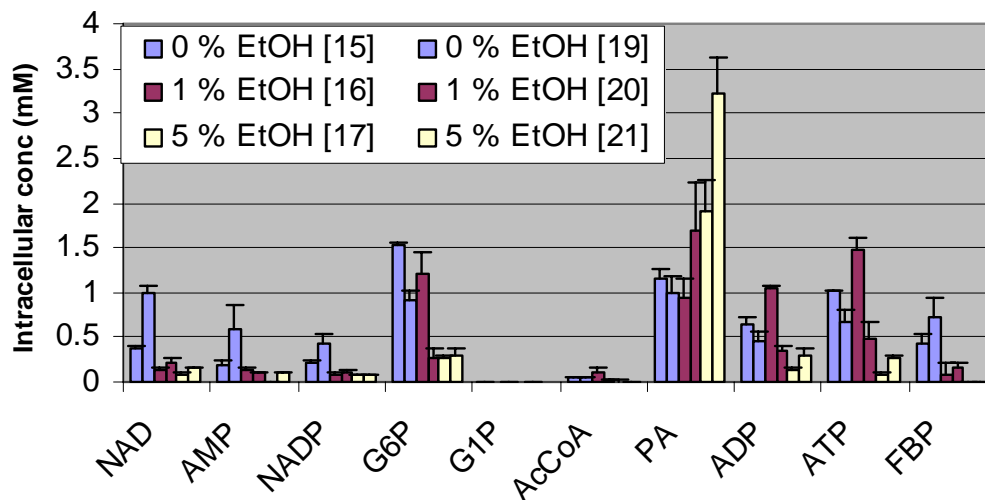
WT chemostat analysis



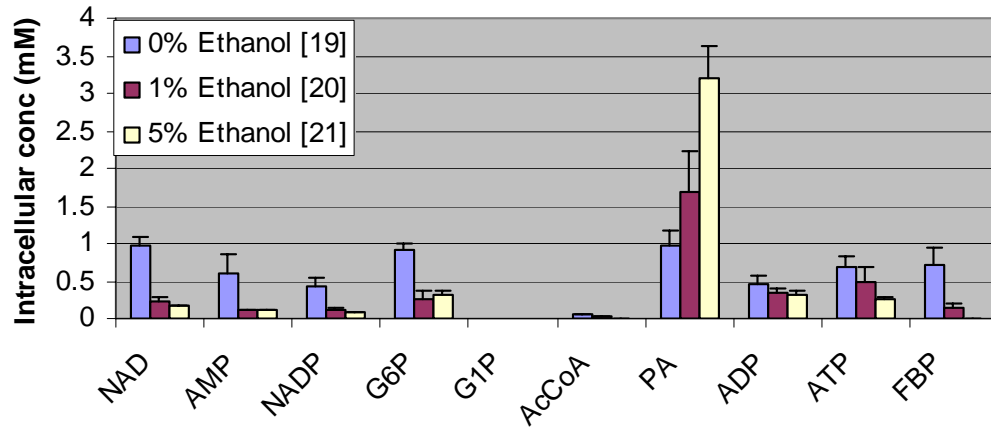
EA Chemostat analysis



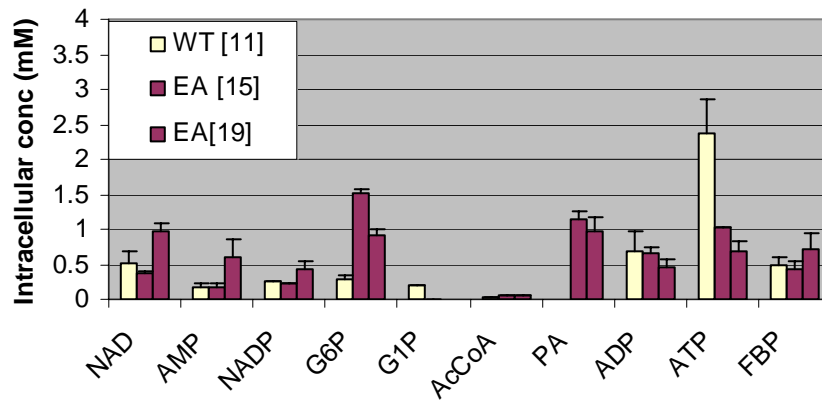
EA chemostat analysis



EA Chemostat analysis



WT vs EA (0% Ethanol)



WT vs EA (1 % Ethanol)

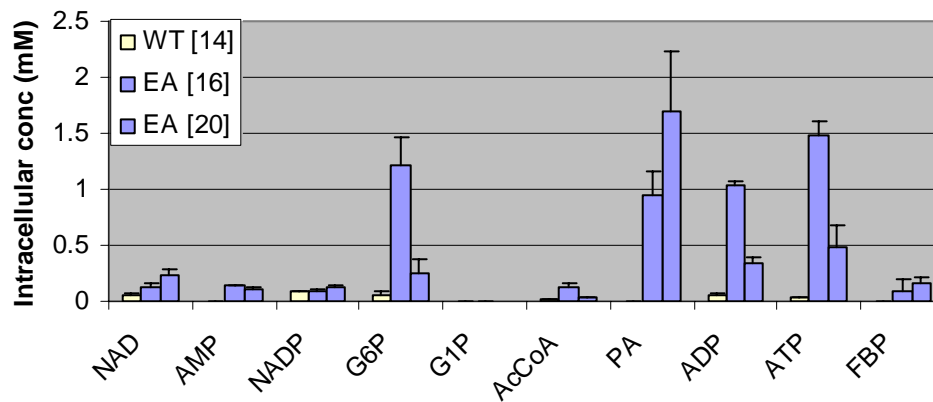


Table I: Product concentrations and carbon recovery of the wild-type (WT) and ethanol-adapted (EA) *Clostridium thermocellum* strains in the continuous culture at a dilution rate $D = 0.05 \text{ h}^{-1}$ and $T = 55^\circ\text{C}$ and at different exogenous ethanol concentrations after 95 % turnover. Numbers in the parentheses represent standard deviations from the duplicate runs.

Parameter	Strain							
	WT				EA			
	Exogenous Ethanol (% w/v)				Exogenous Ethanol (% w/v)			
	0	0.5	1	5 ^a	0	1	5	8 ^a
Extracellular Glucose (mM)	0.1 (0.05)	0.1 (<0.05)	0.2 (0.05)	1.4	0.5 (0.03)	0.4 (0.2)	0.5 (0.08)	0.3
Lactate (mM)	1.6 (0.3)	1.9 (0.7)	2.2 (0.1)	4	2.9 (0.5)	2.2 (0.1)	2 (0.2)	8.1
Acetate (mM)	6.6 (1.6)	7.2 (1.5)	7.3 (1)	2.4	5.8 (0.1)	5.8 (1.2)	6.5 (0.5)	1.7
Endogenous Ethanol (mM)	21.6 (1.3)	- ^b	- ^b	- ^b	25 (1.5)	- ^b	- ^b	- ^b
Carbon Recovery (%)	76 (4)	- ^c	- ^c	- ^c	92 (3)	- ^c	- ^c	- ^c

^a Sampling was done after 95% turnover (under unsteady state condition) as the cell density was declining.

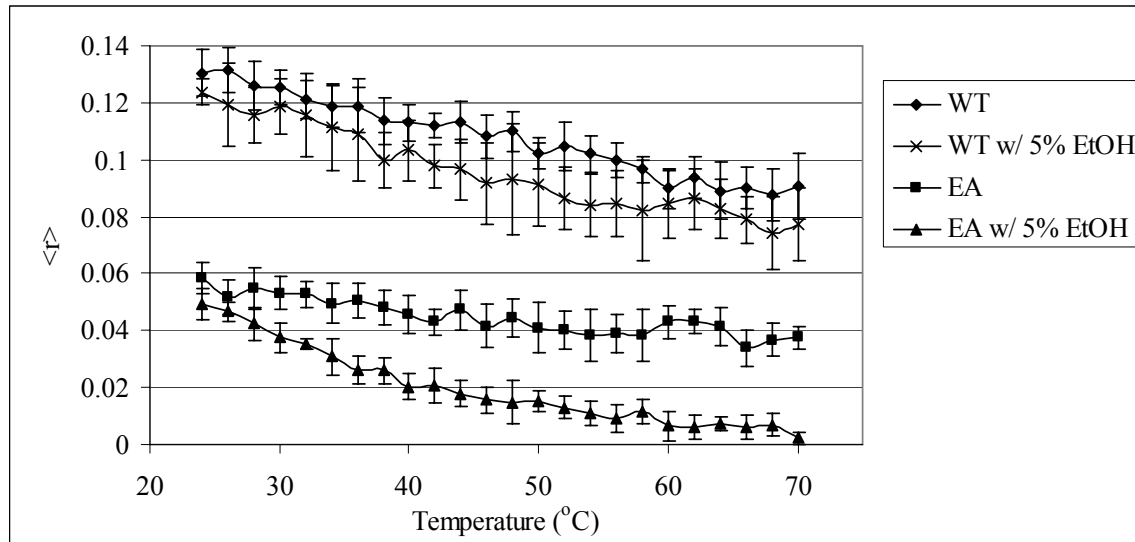
^b Not reported due to large interference from exogenous ethanol, a limitation in our experiments

^c Not calculated because of the lack of reliable ethanol concentrations

Objective 2: Membrane fluidity changes between the wild type and an ethanol-adapted strain of *C. thermocellum* as a function of exogenous ethanol.

Preliminary data was collected on the anisotropy of *C. thermocellum* cells as a function of temperature to ascertain the efficacy of installing the membrane fluidity probe. The saponification and methylation procedures to form fatty acid methyl esters (FAMES) from *C. thermocellum* whole cells was revised from the standard protocol to a new method that increases yield by up to three times (Figure 1). The revised method produces yields that are on par with expected literature values for lipid content on a dry cell weight basis. Studies to determine the structure of the fatty acyl chains have been conducted. Picolinyl esters, formed through transesterification reactions, provide mass spectra with characteristic fragmentation patterns for hydrocarbon chains. From these studies the principle component of *C. thermocellum*'s membrane was identified as *i*-16:0 (Figure 2) followed by *n*-16:0 (Figure 3). Other fatty acids identified were *i*-17:0, *n*-17:0, *i*-18:0, and *n*-18:0

Anisotropy results for *Clostridium thermocellum* wild-type and ethanol-adapted.



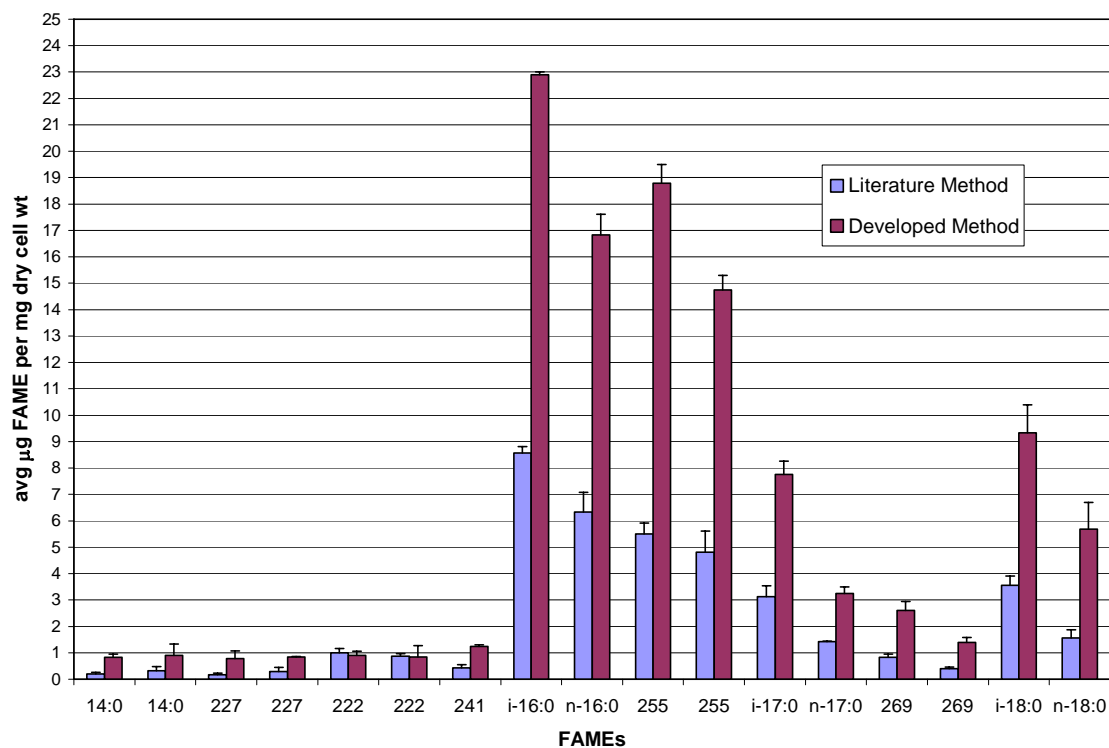
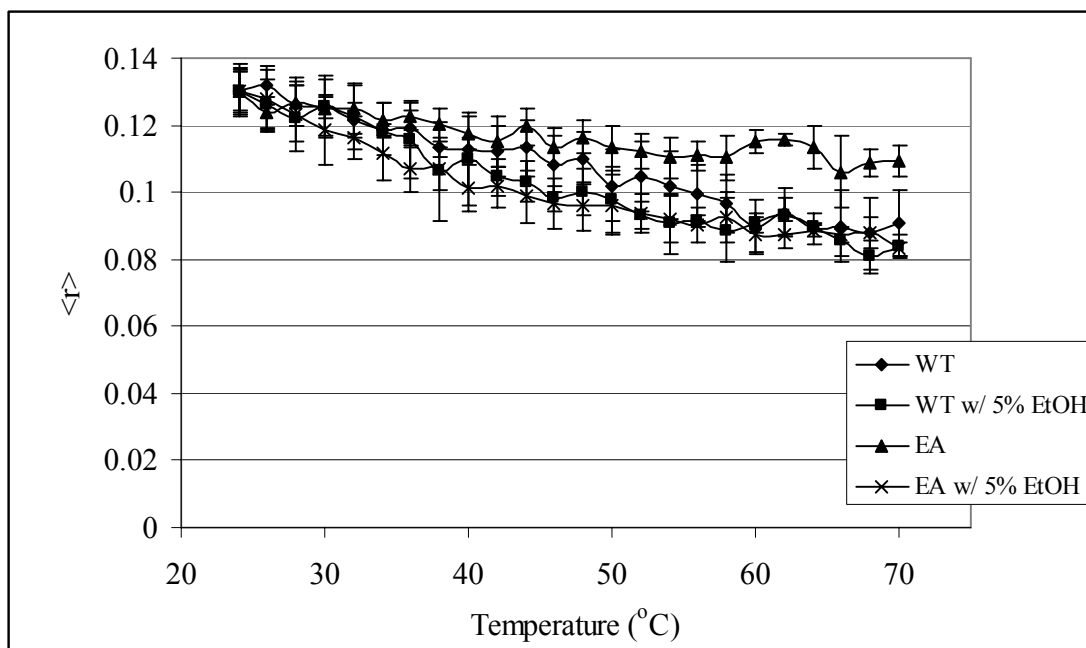
n=3; error bars the pooled stdev of replicates

WT: $-0.001x + 0.1533$; $R^2 = 0.9729$

WT w/ 5% EtOH: $-0.001x + 0.1448$; $R^2 = 0.9435$

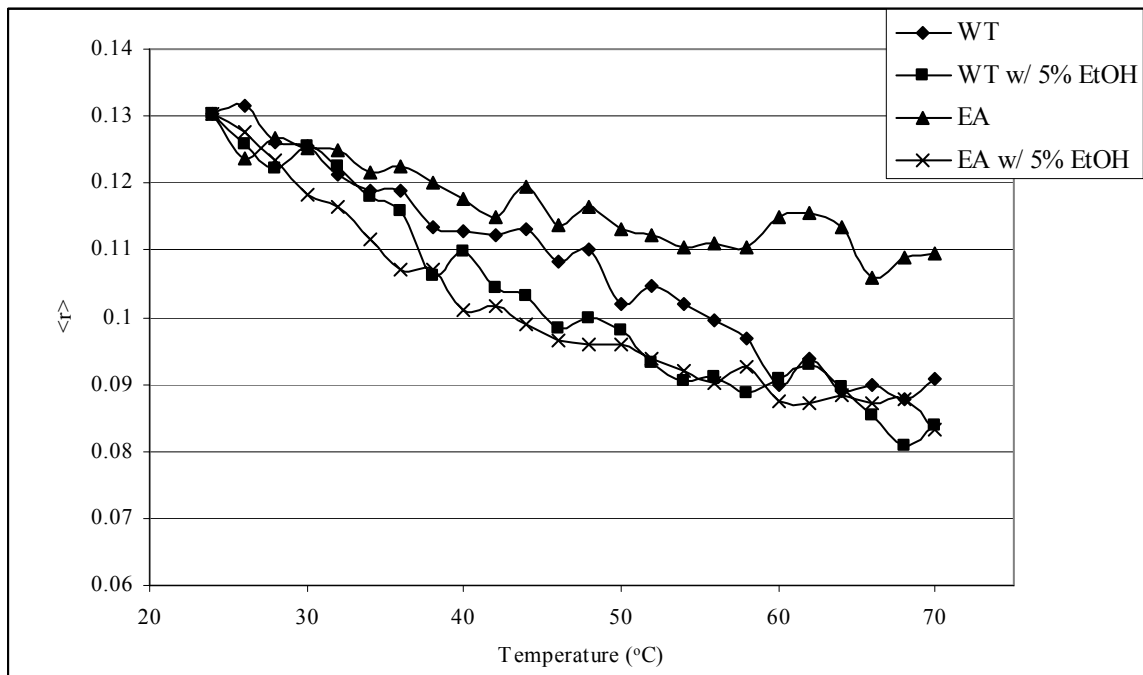
EA: $-0.0004x + 0.0643$; $R^2 = 0.8295$

EA w/ 5% EtOH: $-0.0009x + 0.0639$; $R^2 = 0.9098$



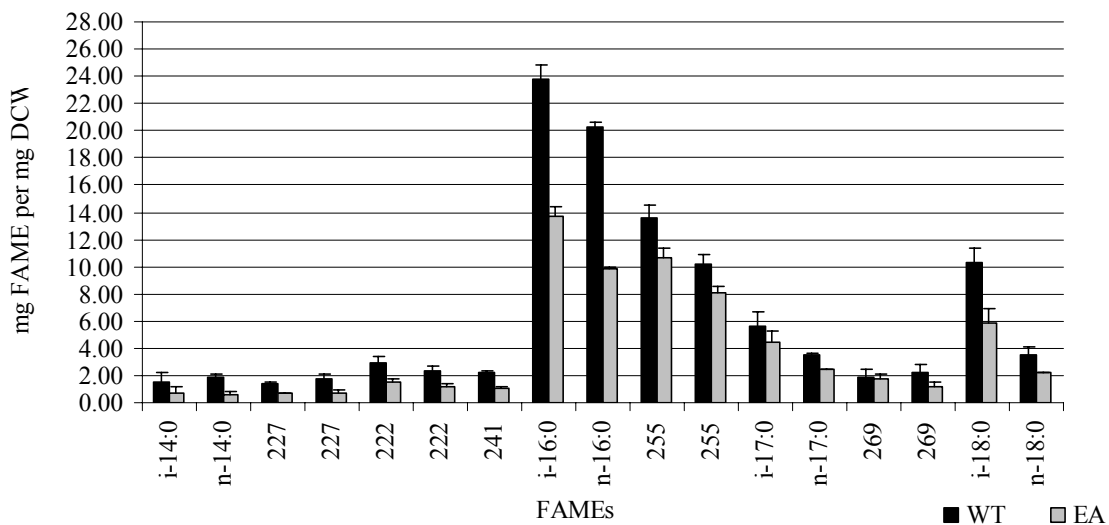
Normalized graph: normalizes anisotropy to WT samples.

i.e. $\langle r \rangle = ((\text{avg } r) + (\Delta \text{WT} - r @ 24^\circ\text{C}))$



Fatty acid profiles

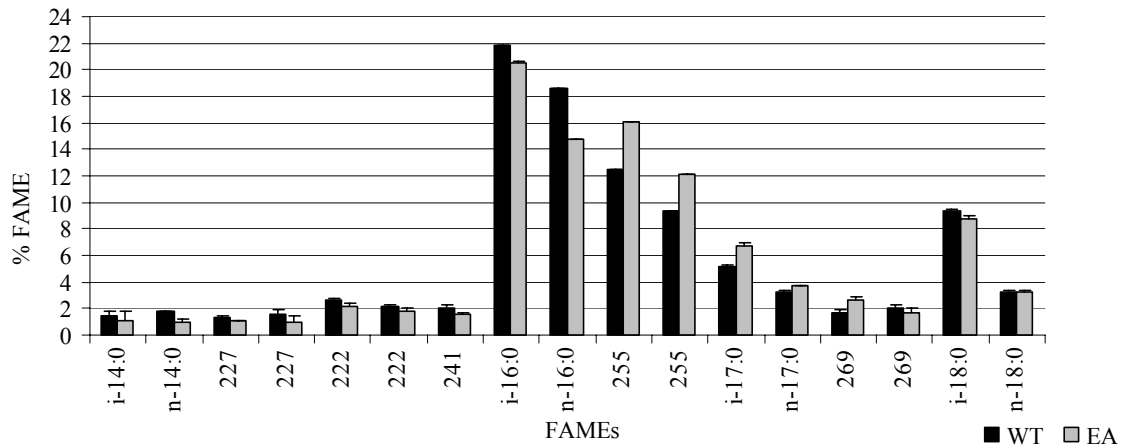
Comparison between *C. thermocellum* WT and EA fatty acid profiles



n=3; error bars are pooled stdev of replicates

t-test: Significant difference (95%) found between: n-14:0, 227, 227, 222, 222, i-16:0, n-16:0, 255, 255, and i-18:0

Comparison between *C. thermocellum* WT and EA fatty acid percentages



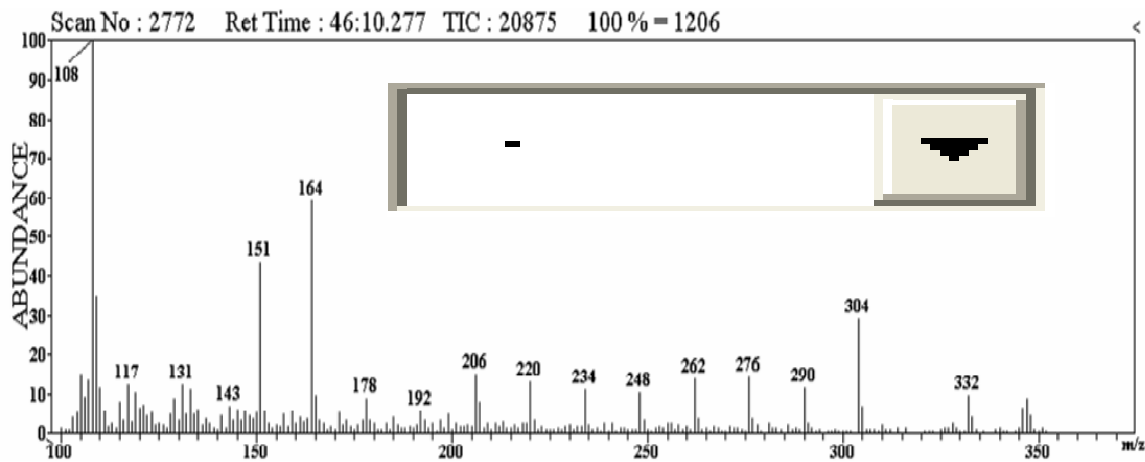
n=3; error bars are RSD of replicates

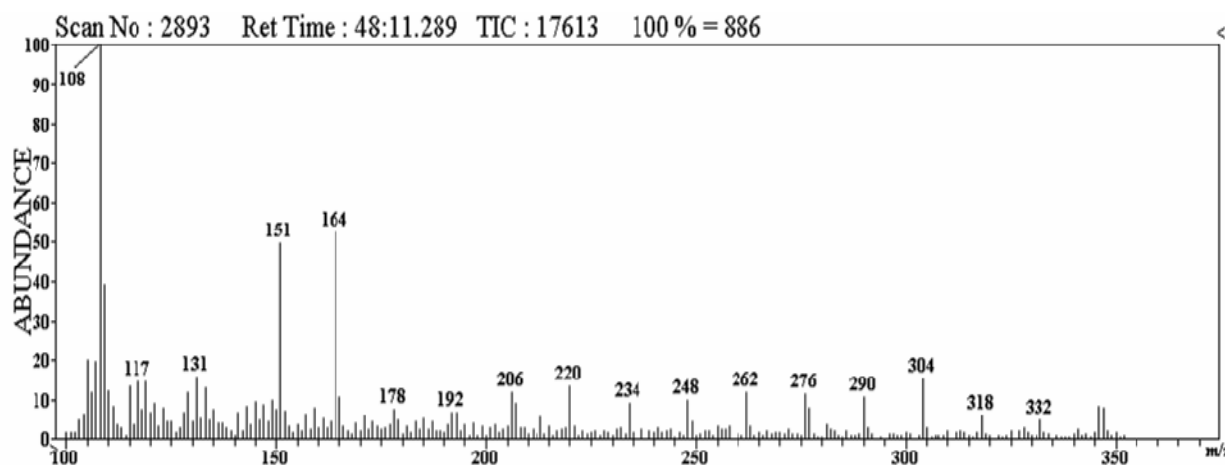
Results: t-test: Significant difference (95%) found between: n-14:0, 227, 222, i-16:0, n-16:0, 255, 255, i-17:0, n-17:0, 269, and i-18:0

- **WT: 10.9% lipid/DCW EA: 6.6% lipid/DCW**
- **EA has greater % long chain fatty acids**

Herrero:

- Results differ from Herrero et al.
 - EA increase in <C14 fatty acids, unsaturation
 - Increase in n- and a-, reduction in i- branched





The concept that microbes modify their membrane composition in response to environmental stress has been well established. The theory of homeoviscous adaptation (cells modify their membranes in order to maintain an optimal degree of fluidity) has been supported by studies relating fatty acid profiles to solvent insult. Among the literature there are numerous papers reporting relationships between fatty acid chain length, ethanol exposure, and membrane fluidity (Alexandre et al. 1994b; Gutierrezruiz et al. 1995; Tymczynsyn et al. 2005). Studies with ethanol-tolerant bacteria have shown that membrane fluidity increases due to growth or exposure to ethanol.

The accepted standard is that bacteria increase the number of unsaturated and short chain fatty acids in response to ethanol insult. Studies conducted by Herrero found that ethanol-adaptation increases unsaturated and short-chain fatty acids. Unsaturated and shorter chain fatty acids provide the membrane with a greater degree of fluidity compared to saturated alkyl chains. The conclusion drawn was that *C. thermocellum* responds to ethanol treatment by increasing membrane fluidization (Herrero et al. 1982). Their conclusion was based on the theory that ethanol has a rigidifying effect on the membrane. This was based on partition coefficient studies with liposomes and branched-chain alcohols (Jain and Wray 1978).

Our fatty acid results counter previously published studies on ethanol-adapted *C. thermocellum* strains. The fatty acid data presented here shows that ethanol-adapted *C. thermocellum* strain increases the percentage of longer chain fatty acids when compared to wild-type strains. In addition ethanol insult did not promote the formation of mono-unsaturation fatty acids. The shift to longer, saturated-chain fatty acids reduced the native fluidity of the membrane.

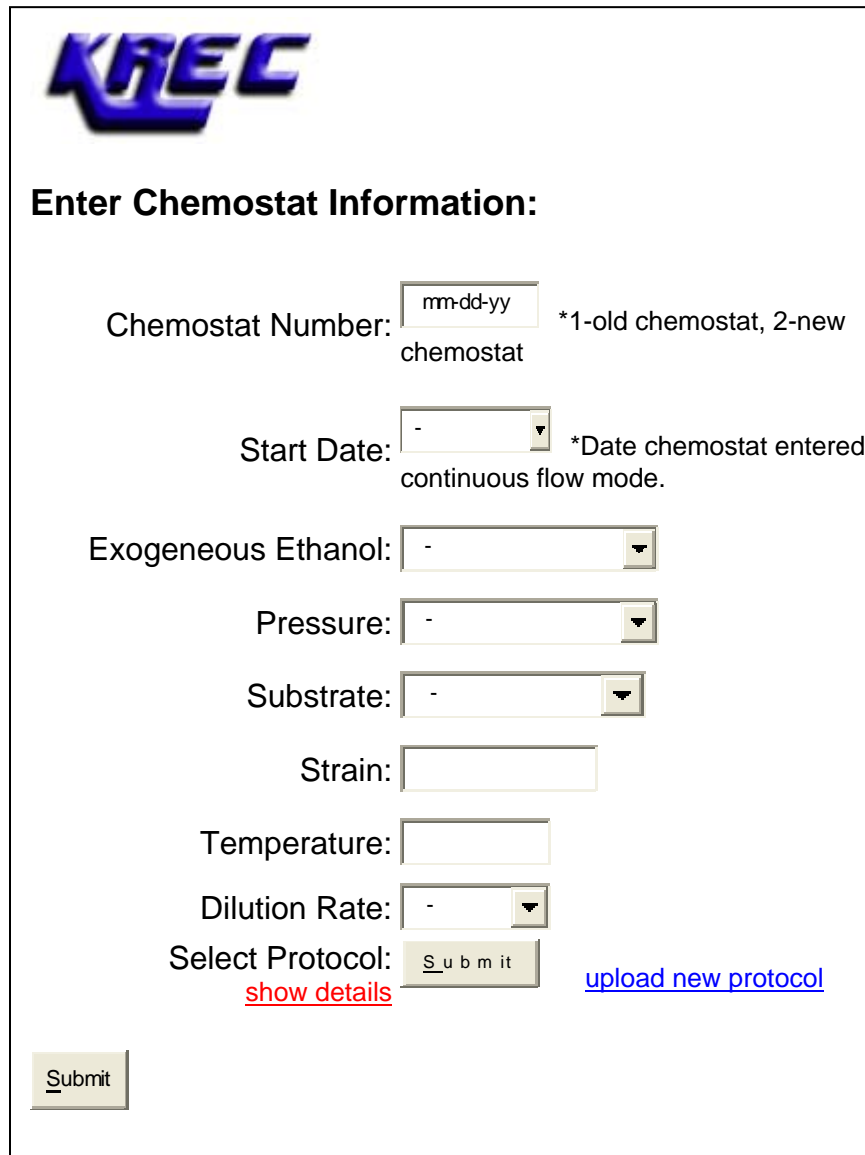
The anisotropy data presented within this manuscript supports findings that ethanol exposure increases membrane fluidity (Konopasek et al. 2000). According to steady-state fluorescence anisotropy experiments exposure to ethanol increases the fluidity of both *C. thermocellum* WT and EA strains. Ethanol-adapted cells when in the presence of ethanol acquire increased fluidity to a level that is consistent to that of wild-type membrane fluidity.

We conclude that ethanol tolerance for *C. thermocellum* is the result of membrane adaptation that reduces fluidity, allowing the integrity of the membrane to be maintained during growth in the presence of ethanol. This study provides evidence of a new route for bacterial survival due to adaptation to exogenous solvent.

Understanding the mechanism of membrane adaptation by *C. thermocellum* to acquire tolerance to ethanol could be utilized to develop cultures that exhibit resistance to higher concentrations of ethanol. Fermentation with these adapted strains could reduce cell mortality due to molecular toxicity and therefore be used for production of greater quantities of ethanol per batch culture. This in-turn could lead to more cost-effective ethanol production and a step closer to consolidated bioprocessing (CBP).

Objective 3: Correlate the pathway changes and membrane fluidity with environmental treatments to gain a mechanistic understanding of cellular adaptation in an ethanol-tolerant organism.

Graduate Student 1 (BAE): has created a web-based database so that all investigators can share data input and retrieval. Tables are being created and filled as data are generated.



The image shows a web-based data entry form for KREC. At the top left is the KREC logo. Below it, the heading "Enter Chemostat Information:" is displayed. The form contains several input fields and buttons:

- Chemostat Number:** A text input field with a placeholder "mm-dd-yy" and a note "*1-old chemostat, 2-new chemostat".
- Start Date:** A date selection dropdown menu with a note "*Date chemostat entered continuous flow mode."
- Exogeneous Ethanol:** A dropdown menu with a hyphen "-" as the selected option.
- Pressure:** A dropdown menu with a hyphen "-" as the selected option.
- Substrate:** A dropdown menu with a hyphen "-" as the selected option.
- Strain:** A text input field.
- Temperature:** A text input field.
- Dilution Rate:** A dropdown menu with a hyphen "-" as the selected option.
- Select Protocol:** A button labeled "Submit" (with the letters spaced out) and a link labeled "show details" in red text.
- upload new protocol** in blue text.
- A large **Submit** button at the bottom left.

Figure 1: Example data entry table from database.

1. : Database forms were completed for membrane fluidity experiments, so complete database is constructed. Students/staff were trained on data entry.
2. Database forms were completed for membrane fluidity experiments, so complete database is constructed. Students/staff were trained on data entry

3. . Complete database is constructed. Students/staff were trained on data entry. Data export options improved.

Post-Doc (CME): Nine chemostat experiments have been run varying dilution rate and exogenous ethanol concentration in a pressurized reactor. Exogenous ethanol concentrations have been changed during the run, a new equilibrium conditions attained, and samples taken for further analysis by CE/MS.

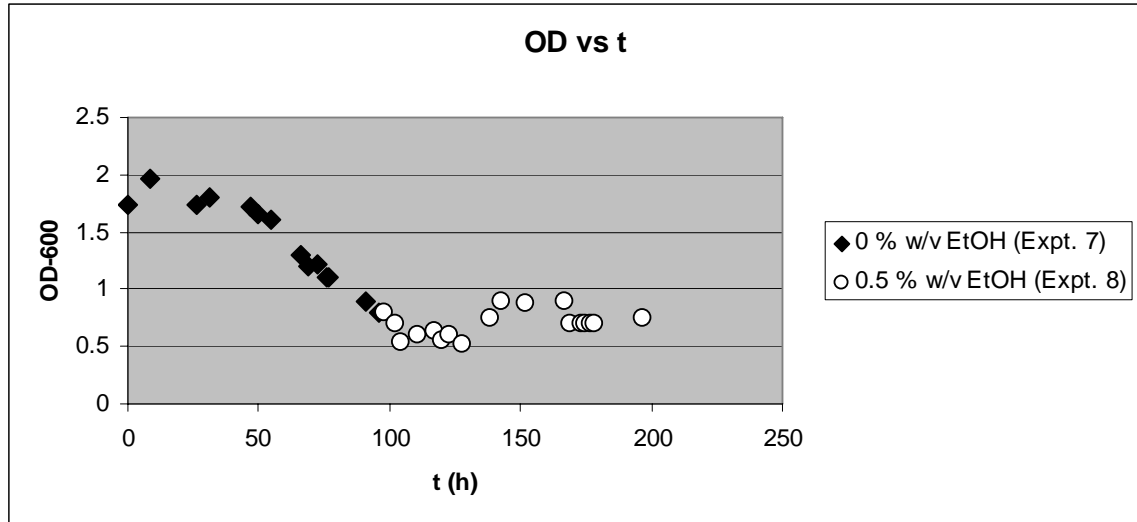


Figure 2: Optical densities for chemostat with 0 and 0.5% w/v exogenous ethanol for growth of *Clostridium thermocellum* on cellobiose in continuous flow mode.

The continuous cultures of the WT and EA strains were performed at a range of exogenous ethanol concentrations (0 to 8% w/v ethanol). It was found in the batch culture experiments that the maximum growth rates of these two strains were different and that optimum growth temperatures of these two strains were also different that is 60°C for the WT and 55°C for the EA (data not shown). At $T = 55^{\circ}\text{C}$, WT manifested ethanol tolerance up to 1% w/v ethanol beyond which the growth was significantly inhibited while EA was found to be reliably growing and tolerant up to 6% w/v ethanol (data not shown). In our culture experiments we observed that the morphology of WT and EA were quite different. Moreover, in the preliminary results we also observed that the membrane structure and fatty acid composition of the WT and EA were different and that these two strains differentially expressed membrane proteins (Williams et al., 2006).

Based on the above preliminary results we expected that the metabolism of the wild-type and ethanol-adapted will be different. During the anaerobic fermentation of cellobiose by *C. thermocellum* strains, wild-type and ethanol-adapted; cellobiose, glucose, lactate, acetate, and ethanol concentrations were detected and measured where possible.

Microbial Growth and Substrate Utilization

Extent of microbial growth gives us an indication of the growth conditions *i.e.* response to environmental stress which in our case is exogenous ethanol. It is evident from **Fig. 1** that in addition to the growth in non-exogenous ethanol continuous cultures (control experiment), microbial growth was also observed in ethanol challenging conditions. Moreover, the presence of metabolic end-products suggested that the fermentation of cellobiose continued in exogenous ethanol runs (*see Table I*). In the case of WT strain, very similar cell densities were observed for exogenous ethanol treatments up to 1% w/v ethanol that is in the range 0.6 – 0.7 gDCW/L but at 5% w/v ethanol the cell density after 95% turnover reached zero (*see Fig. 1*). However, EA strain manifested greater resistance to ethanol and maintained growth consistently for ethanol concentrations up to 5% w/v but the cell density in the 8% w/v ethanol run reached zero after 95% turnover (*see Fig. 1*).

Cellobiose was nearly completely consumed for both the *C. thermocellum* strains at exogenous ethanol concentration below 1% w/v ethanol as shown in **Fig. 2**. However, cellobiose started to accumulate at higher exogenous ethanol levels. Although the case of WT and EA cells respectively at 5% and 8% w/v ethanol is obvious because of the decreasing cell density that reached zero after 95% turnover (refer to **Fig. 1**) that is an

unsteady state situation but interestingly EA strain even with significant microbial growth at 5% w/v ethanol failed to completely utilize cellobiose.

Since cellobiose can also be converted externally to glucose (Strobel, 1995) therefore although extracellular glucose concentrations shown in **Table I** are low (< 6% of feedstock hexose) but these numbers are not insignificant and so merit further analysis. A comparison of the WT and EA strains suggest that similar extracellular glucose concentrations were observed. Compared with WT, EA expressed marginally higher glucose concentrations in majority of the cultures except at 5% w/v ethanol.

The *overall* average experimental cell growth yield ($Y_{X/S} = \Delta X / \Delta S$, g DCW g⁻¹ cellobiose consumed) including the exogenous ethanol experiments was calculated after removing the extracellular glucose concentration from the cellobiose consumed. Both the strains (WT, 0 to 1% w/v ethanol; EA, 0 to 5% w/v ethanol) manifested similar cell growth yields where WT managed somewhat higher growth yield ($Y_{X/S}^{WT} = 0.18 \pm 0.01$ g DCW g⁻¹ cellobiose consumed and $Y_{X/S}^{EA} = 0.16 \pm 0.01$ g DCW g⁻¹ cellobiose consumed). Based on the very small standard deviations (*see Table I*), it is interesting to note that the cell growth yields in the respective strains were largely independent of exogenous ethanol treatment.

Fermentation Products and Selectivity

Product yields ($Y_{P/S} = \Delta P / \Delta S$, mmol of product formed per mmol of substrate consumed) for the two strains is shown in **Figs. 3, 4 and 5**. Considering the runs where microbial growth did sustain *i.e.*, not including the highest exogenous ethanol runs that is 5% for WT and 8% for EA, the lactate yields for the two strains WT and EA were very low that is the molar carbon flow toward the total production of lactate was less than 8%.

A comparison of the WT and EA suggests that lactate yields were very similar except for EA in the non-exogenous ethanol run (*see Fig. 3*) where highest yield was observed but was not statistically different.

Acetate yields are shown in **Fig. 4**, it is evident from the responses of the WT and EA that acetate yields largely remained unchanged and were found to be in the range 0.6 to 0.8 mmol of acetate per mmol of cellobiose consumed. As a summary, the total organic acid production (lactate and acetate together) in the two strains were very similar under various ethanol treatments.

Interestingly, during the highest exogenous ethanol experiments, which is 5% for WT and 8% w/v ethanol for EA the lactate values increased with a concomitant decrease in the acetate values (*see Table I*).

Although ethanol was not measured in the exogenous ethanol experiments, but presented in **Fig. 5** is the yield and selectivity of endogenous ethanol (bioethanol produced) during the non-exogenous ethanol run *i.e.*, our control experiment. Here, WT expressed $Y_{E/S}^{WT} = 2.1 \pm 0.2$ mmol ethanol mmol⁻¹ cellobiose consumed, but surprisingly EA expressed slightly higher ethanol yield *i.e.* $Y_{E/S}^{EA} = 2.7 \pm 0.1$ mmol ethanol mmol⁻¹ cellobiose consumed. In congruence with ethanol yield, the molar ratio *i.e.* ethanol/(lactate+acetate) was also slightly higher with EA when compared with WT (*see Fig. 5*).

Carbon Recovery

In our chemical analysis, gases, CO₂ and H₂ were not measured. However, the gas concentrations were predicted by employing metabolic flux analysis. The calculated percentage carbon recoveries are presented in **Table I**. Because of the lack of ethanol

concentrations from the exogenous ethanol experiments (*refer to materials and methods* section on enzymatic assays) only percentages corresponding to representative non-exogenous ethanol cultures are presented. WT expressed carbon recovery percentage of 76 ± 4 but EA on the contrary, expressed significantly high percentage *i.e.* 92 ± 3 .

Table I: Product concentrations and carbon recovery of the wild-type (WT) and ethanol-adapted (EA) *Clostridium thermocellum* strains in the continuous culture at a dilution rate $D = 0.05 \text{ h}^{-1}$ and $T = 55^\circ\text{C}$ and at different exogenous ethanol concentrations after 95 % turnover. Numbers in the parentheses represent standard deviations from the duplicate runs.

Parameter	Strain							
	WT				EA			
	Exogenous Ethanol (% w/v)				Exogenous Ethanol (% w/v)			
	0	0.5	1	5 ^a	0	1	5	8 ^a
Extracellular Glucose (mM)	0.1 (0.05)	0.1 (<0.05)	0.2 (0.05)	1.4	0.5 (0.03)	0.4 (0.2)	0.5 (0.08)	0.3
Lactate (mM)	1.6 (0.3)	1.9 (0.7)	2.2 (0.1)	4	2.9 (0.5)	2.2 (0.1)	2 (0.2)	8.1
Acetate (mM)	6.6 (1.6)	7.2 (1.5)	7.3 (1)	2.4	5.8 (0.1)	5.8 (1.2)	6.5 (0.5)	1.7
Endogenous Ethanol (mM)	21.6 (1.3)	_b	_b	_b	25 (1.5)	_b	_b	_b
Carbon Recovery (%)	76 (4)	_c	_c	_c	92 (3)	_c	_c	_c

^a Sampling was done after 95% turnover (under unsteady state condition) as the cell density was declining.

^b Not reported due to large interference from exogenous ethanol, a limitation in our experiments

^c Not calculated because of the lack of reliable ethanol concentrations

FIGURE CAPTIONS

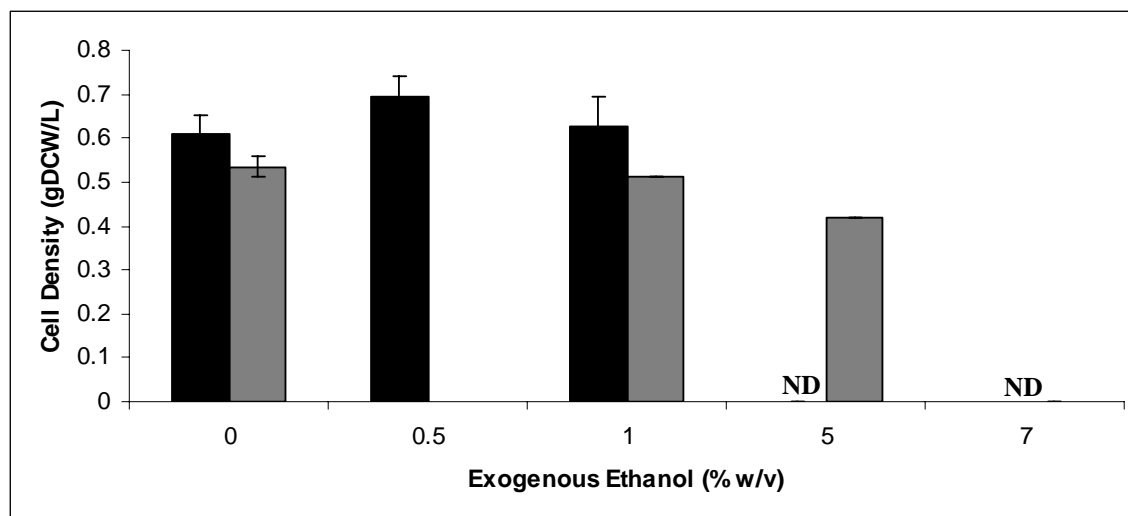
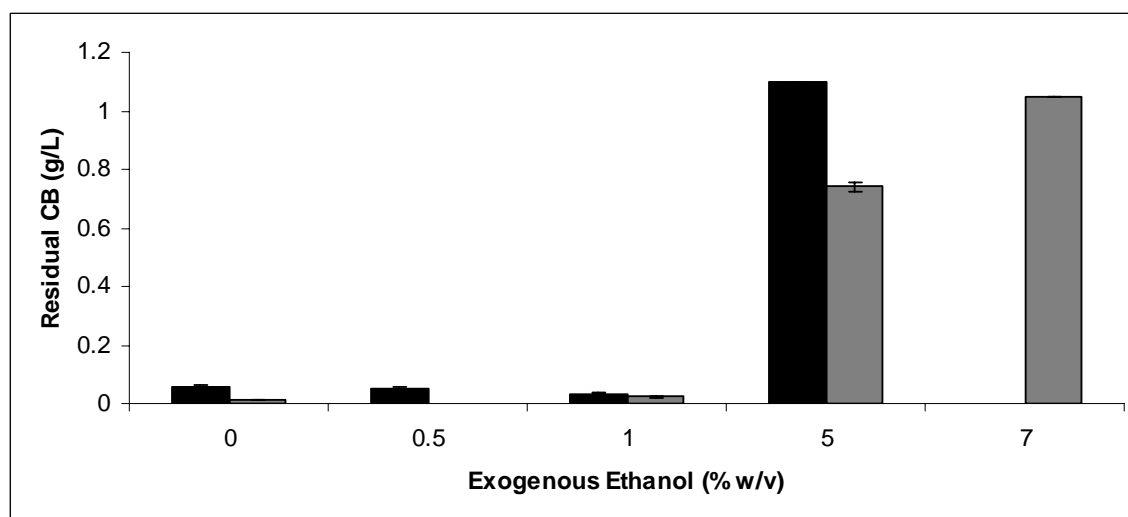
Figure 1: Variation of cell density with exogenous ethanol in the continuous cultures of wild-type (black bars) and ethanol-adapted (gray bars) *C. thermocellum* strains. $T = 55^{\circ}\text{C}$ and $D = 0.05 \text{ h}^{-1}$.

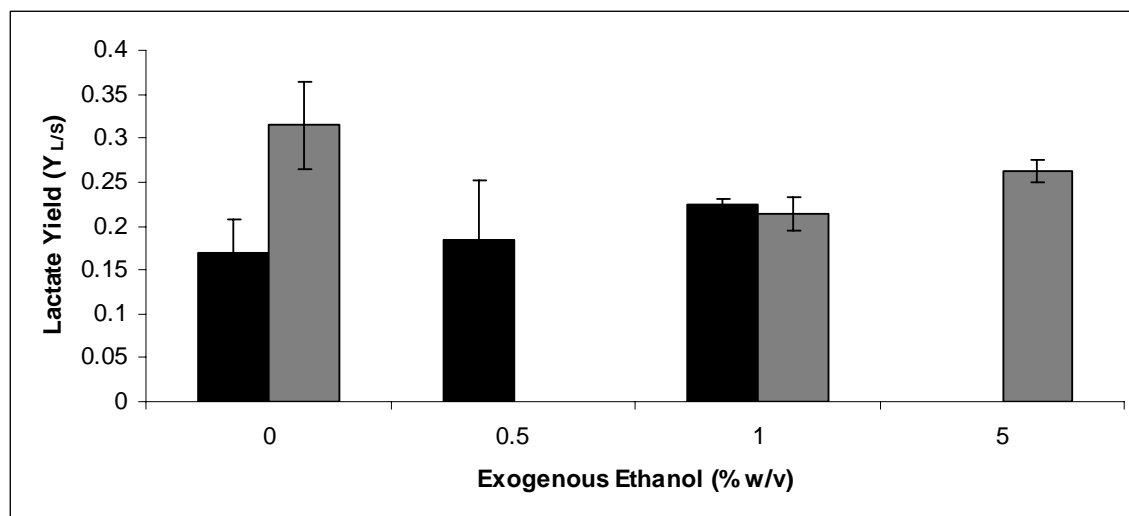
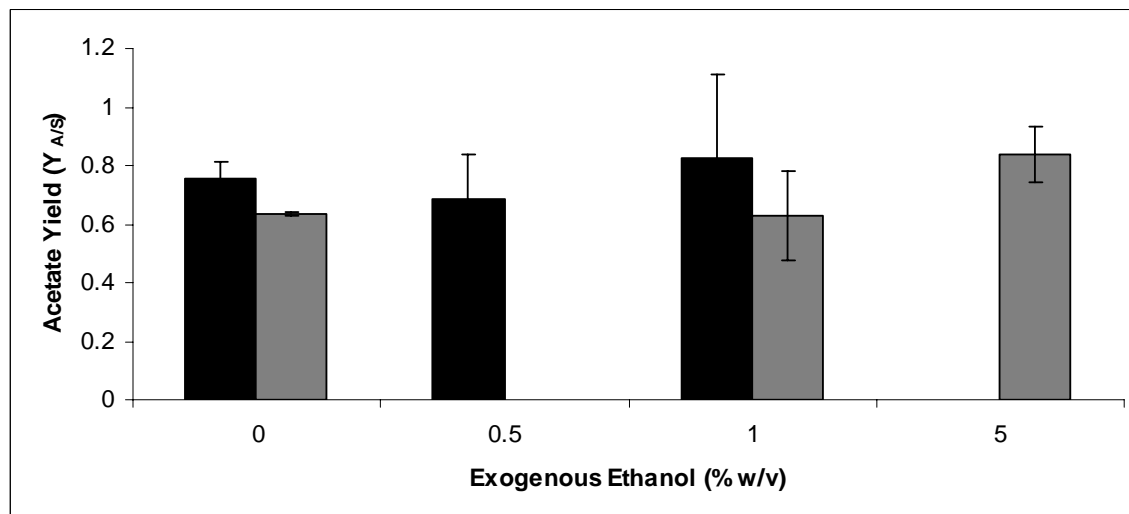
Figure 2: Residual cellobiose concentration as a function of exogenous ethanol in the continuous cultures of wild-type (black bars) and ethanol-adapted (gray bars) *C. thermocellum* strains. $T = 55^{\circ}\text{C}$ and $D = 0.05 \text{ h}^{-1}$.

Figure 3: Lactate yield Y_{LS} (mol lactate / mol cellobiose consumed) as a function of exogenous ethanol in the continuous cultures of wild-type (black bars) and ethanol-adapted (gray bars) *C. thermocellum* strains. $T = 55^{\circ}\text{C}$ and $D = 0.05 \text{ h}^{-1}$.

Figure 4: Acetate yield Y_{AS} (mol acetate / mol cellobiose consumed) as a function of exogenous ethanol in the continuous cultures of wild-type (black bars) and ethanol-adapted (gray bars) *C. thermocellum* strains. $T = 55^{\circ}\text{C}$ and $D = 0.05 \text{ h}^{-1}$.

Figure 5: Ethanol yield Y_{ES} (mol ethanol / mol cellobiose consumed) on primary axis as a function of exogenous ethanol (black bars) with ethanol / (lactate + acetate) (mol/mol) on the secondary axis (gray bars) in the continuous cultures of wild-type and ethanol-adapted *C. thermocellum* strains. $T = 55^{\circ}\text{C}$ and $D = 0.05 \text{ h}^{-1}$.

**Figure 1****Figure 2**

**Figure 3****Figure 4**

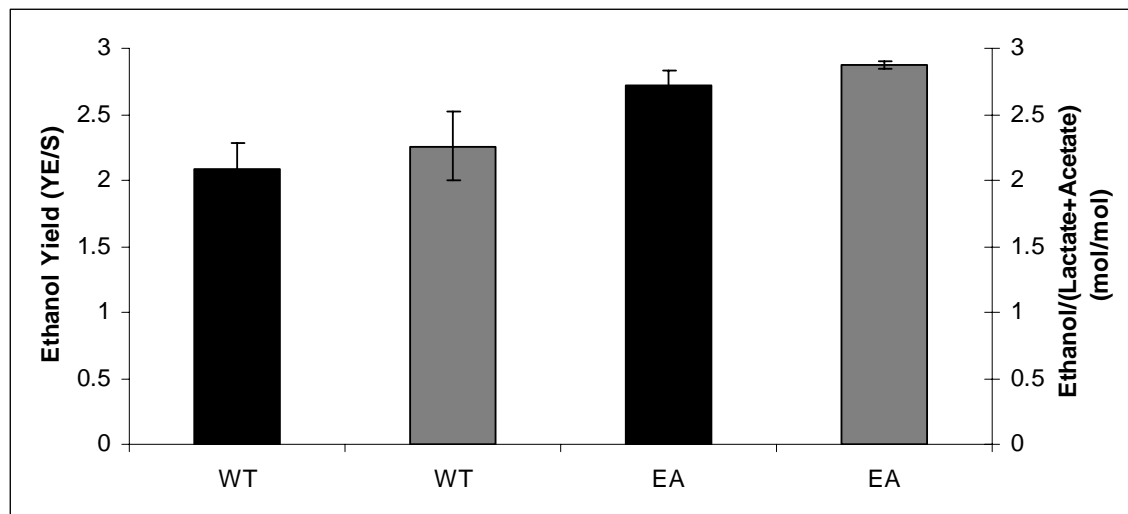


Figure 5

Publications / Presentations:

4. Explanation of Variance: None
5. Plans for Next Quarter: None (grant ended)

Patents: N/A

Publications / Presentations:

Adotey, B., S.E. Nokes, and H.J. Strobel. 2006. Metabolic model development for *Clostridium thermocellum*: a thermophilic anaerobe capable of converting lignocellulose to ethanol. Presented at the 2006 ASABE Annual International Meeting July 9-12, 2006 Portland, OR. ASABE No. 067005.

Adotey, B., Sue E. Nokes, Herbert J. Strobel. 2007. Metabolic Flux Analysis of *Clostridium thermocellum*. Presentation to the ASABE meeting, Minneapolis, MN, June 17-21, 2007.

Michael D. Timmons; Herbert J. Strobel; Barbara L. Knutson; Sue E. Nokes; Bert C. Lynn . 2007. Lipid Composition Comparison and Structural Analysis from *Clostridium thermocellum* Wild-type and Ethanol-adapted Strains; 55th ASMS Conference on Mass Spectrometry; June 3 - 7, 2007, Indianapolis, Indiana; poster WPZ 422.

A. P. Thakur; H. J. Strobel; B. L. Knutson; S. E. Nokes and B. C. Lynn, 2007, Methods Development of Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry (CE-ESI-MS) for Metabolic Comparison of Wild Type and Ethanol Adapted Strains of *Clostridium thermocellum*, 55th American Society for Mass Spectrometry (ASMS) conference on Mass spectrometry 2007, Indianapolis, IN, USA, Poster TLP 212.

Satyakrishna Jujjuri, Barbara L. Knutson, Sue E. Nokes, Herbert J. Strobel, and Bert C. Lynn. Metabolic profile of wild-type and ethanol-adapted *Clostridium thermocellum* strains in exogenous ethanol continuous cultures. Presented to the ACS Symposium, Aug, 2007. Boston, MA.

Michael D. Timmons, Barbara L. Knutson, Sue E. Nokes, Herbert J. Strobel, and Bert C. Lynn. Analysis of composition and structure of *C. thermocellum* membranes from wild-type and ethanol-adapted strains. Manuscript submitted to Biotechnology and Bioengineering, January, 2008